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THE EFFECTS OF WEATHERED CRUDE OIL FROM THE M/T ALVENUS SPILL ON EGGS AND YOLK-SAC LARVAE OF RED DRUM (*SCIAENOPS OCELLATUS*)

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ABSTRACT The British tanker M/T ALVENUS ran aground 16.1 km south of Cameron, Louisiana, on 30 July 1984. An estimated 10,157 MT of Venezuelan crude oil were spilled into the Gulf of Mexico. Approximately 2,700 MT of the heavy viscous oil impacted beaches and an additional 1,360 MT remained in the subtidal areas of west Galveston Island, about 160 km southwest of the accident site. Red drum, which spawn in the Gulf of Mexico in the fall, could have been seriously impacted by oil concentrations potentially lethal to eggs or larvae. The impact of weathered crude oil on the survival, growth, and morphological development of red drum eggs and larvae was assessed in the laboratory. Equal numbers of eggs were randomly assigned to one of six treatments of weathered crude oil (control, 50, 100, 500, 1,000 and 2,000 mg/l) and observed through the yolk-sac stage. There were no differences in mean survival, length of surviving larvae, and frequency of morphological abnormalities among treatments ($\alpha \leq 0.05$). In addition, the frequency of spinal deformity and abnormal mouth development was low in all treatments. The initial chemical composition of the fresh crude oil and the seasonally warm weather contributed to the natural degradation of the soluble toxic components.

INTRODUCTION

The British oil tanker M/T ALVENUS ran aground 16.1 km south of Cameron, Louisiana, on 30 July 1984. An estimated 10,157 MT of Venezuelan crude oil were spilled into the Gulf of Mexico. The resulting surface slick drifted in a southwestern direction for 3 days before making landfall. The path of the slick passed the mouths of three coastal passes, Sabine Pass, Rollover Pass, and the Galveston Bay entrance. Approximately 2,700 MT of oil impacted beaches and an additional 1,360 MT remained in the subtidal areas of Galveston Island, about 160 km southwest of the accident site. In the heavily impacted areas of the west end of the island, submerged oil extended 6.0 to 15.3 m offshore and coated the bottom with a layer 15.2 to 20.3 cm deep.

The occurrence of the oil spill coincided with the spawning season of red drum (*Sciaenops ocellatus*). Red drum is a recreationally and commercially important species distributed along the Atlantic and Gulf of Mexico coasts of the U.S., ranging from New York to Texas. Red drum spawn along the Texas coast beginning in mid-August, peaking in late August and early September, and extending to November (Perret et al. 1980). Spawning usually occurs in or around mouths of passes and adjacent offshore waters (Perret et al. 1980). During the spawning season large adult red drum are sometimes numerous along the beachfronts (McEachron 1980). Larval and postlarval redfish occupying these habitats range in size from 4.0 to 12.0 mm SL (Pearson 1929, Sabins 1973, and Guillen 1983).

Based on the path of the surface slick, known spawning season, and the distribution of eggs and larvae, red drum could have been detrimentally impacted by the oil spill through various mechanisms. These mechanisms include physical coating, lethal toxicity, sublethal toxicity, and physiological incorporation (Moore and Dwyer 1974). Laboratory toxicity tests have demonstrated that the embryonic and larval periods are the most sensitive stages in the life history of fishes (McKim 1977). Water soluble components of crude oil have been shown to be toxic to adult and juvenile estuarine organisms (Anderson et al. 1974). Results of experiments conducted on eggs and larvae of Pacific herring (*Clupea harengus*) have demonstrated that water soluble fractions of crude oil increase the occurrence of gross abnormalities and decrease the growth rate of newly hatched larvae (Smith and Cameron 1979). In addition, less obvious deleterious effects have been observed at the cellular level in Pacific herring larvae (Cameron and Smith 1980). Rabalais et al. (1981) reported higher mean mortality and gross abnormalities in red drum eggs and yolk-sac larvae exposed to concentrations of crude oil observed during the Ixtoc I oil spill.

The objective of this study was to evaluate the possible effects of weathered M/T ALVENUS crude oil on the early development of red drum. We define weathered crude oil as oil which remained on the bottom in subtidal and intertidal zones after initial landfall on 4 August 1985. The majority of exposed immature red drum would contact this form of oil during the peak spawning period in September. The acute toxicity and sublethal effects of the weathered crude oil were evaluated using a static bioassay on the eggs and resulting yolk-sac larvae of red drum.

METHODS

Information on the chemical composition of the fresh and weathered crude oil was considered essential for understanding the possible mechanisms of induced mortality and sublethal effects. The cargo holds of the M/T ALVENUS contained two types of crude oil, Merey and Pilon. Crude oil samples were collected from the cargo holds of the M/T ALVENUS and from Jamaica Beach by Conoco Oil Company personnel (Figure 1). Chemical analyses were

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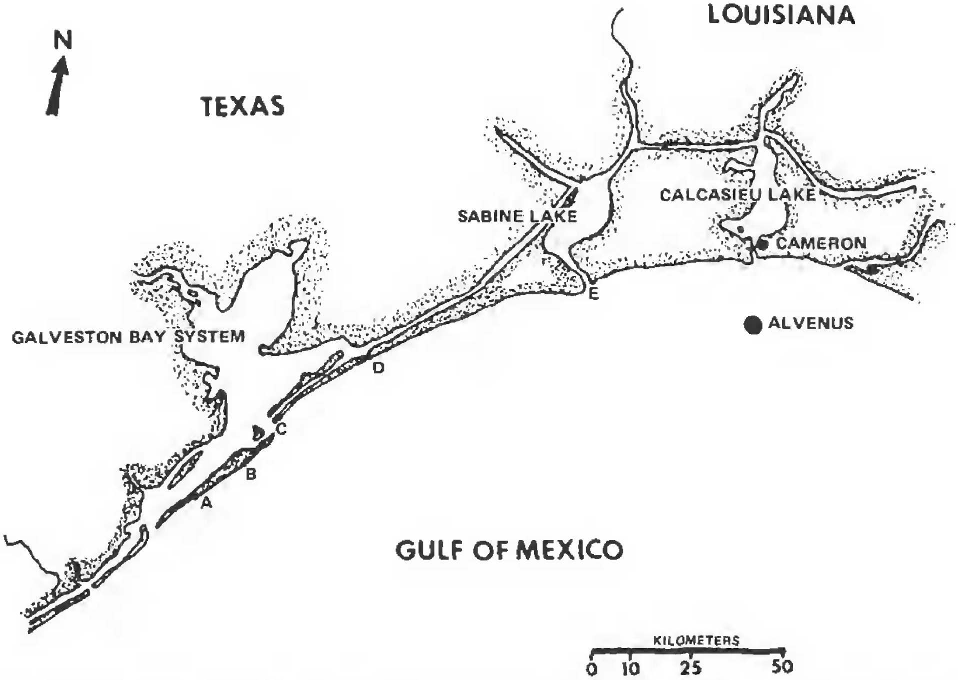

Figure 1. Area affected by the M/T ALVENUS oil spill. (A = Jamaica Beach, B = Galveston seawall, C = Galveston Bay entrance, D = Rollover Pass, E = Sabine Pass).

performed by the Conoco Refining Technical Services Laboratory of Ponca City, Oklahoma (Leeman 1984). The specific gravity at 15.5°C, total sulfur content, heavy metal content, and distillation fractions were determined for both types of fresh crude (ASTM 1984). Except for distillation fractions, the same parameters were measured on weathered oil obtained from Jamaica Beach.

Texas Parks and Wildlife Department (TPWD) personnel collected one surface water sample in the surf zone at Jamaica Beach on 7 August and 8 September 1984. The oil content of these samples was determined by the partition gravimetric technique (Rand et al. 1976).

Samples of weathered crude oil were collected along Galveston seawall by University of Texas Marine Science Institute and TPWD personnel on 7 August and 28 August 1984, respectively (Figure 1). The oil was analyzed using silica gel column chromatography for percent saturated hydrocarbons, aromatic hydrocarbons, nitrogen, sulfur and oxygen compounds, and asphaltenes (Parker 1984). Qualitative observations were made on the composition and quantity of individual compounds within the aromatic fraction using a Perkin Elmer model 910 gas chromatograph equipped with a flame ionization detector (Parker 1984).

A portion of the crude oil obtained on 28 August 1984 was used for the static bioassay (Peltier 1978). Exposure concentrations of 0, 50, 100, 500, 1,000 and 2,000 mg/l crude oil were selected. Three replicate 5-liter McDonald jars containing synthetic seawater prepared from Instant Ocean[1] were used for each treatment concentration. These clear fiberglass cylindrical jars measured approximately 38.1 cm tall with a diameter of 14.3 cm. The oil was added to the water at 2000 hours on 17 September 1984. The salinity, temperature, and dissolved oxygen in all containers were 35 ppt, 26°C, and 6.8 mg/l, respectively. Air stones were used to gently aerate and circulate the water and oil.

Recently fertilized 1-hour-old eggs were obtained from spawning red drum maintained in captivity at the TPWD John Wilson Fish Hatchery at Flour Bluff, Texas. The salinity, temperature, and dissolved oxygen in spawning tanks were 36 ppt, 26°C, and 6.9 mg/l, respectively. Twenty-five eggs were randomly selected and placed into each container on 17 September 1984 between 2130 and 2230 hours. Constant overhead fluorescent illumination was provided and the room temperature was thermostatically maintained at 25°C. Salinity, water temperature, and dissolved oxygen were monitored in each container at 24, 48, and approximately 64 hours after introduction of the eggs. Qualitative observations of the amount of visible floating oil, number of unhatched eggs, and dead and deformed larvae were made. The bioassay was terminated and the yolk-sac larvae

[1] Reference to trade names does not imply endorsement.

were randomly removed between 0950 and 1315 hours on 20 September 1984, approximately 60 to 67 hours postfertilization and 36 to 43 hours posthatching. The number of live and abnormal live larvae were counted. Larvae were considered dead if the body was opaque and if no opercular and/or body movement was observed after tactile stimulation by a small needle. Larvae were considered abnormal if spinal curvature, incomplete mouth and gut development, or other gross deformities were observed. Notochord length (NL) of three randomly selected larvae from each container was measured with an ocular micrometer.

A Kruskal-Wallis one-way analysis of variance was used to test the null hypotheses that percent mortality, percent abnormality of surviving larvae, and mean lengths were equal for all treatments (Daniels 1978). A significance level of $\alpha = 0.05$ was preselected before the analyses.

RESULTS

The oil spilled from the M/T ALVENUS was a heavy viscous crude (Tables 1 and 2). For comparison, an outline of the typical constituents of crude oil, based on distillation fractions, is provided (Table 3) (Morrison and Boyd 1973). The two types of crude oil spilled, Merey and Pilon, exhibited relatively high specific gravities. Lighter alkanes and cycloalkanes were absent. Approximately 70% of the fresh crude oil was composed of insoluble high molecular weight asphaltenes (Tables 1 and 3). In addition, these crude oils contained a large fraction of aromatic hydrocarbons.

The weathered crude oil contained high concentrations of water and sand (Tables 2 and 4). The percentage of high molecular weight asphaltenes per unit of oil was slightly higher in the late August beach sample, relative to the earlier beach sample. In addition, the percentage of total aromatic hydrocarbons per unit of oil had decreased

TABLE 1

Physical and chemical properties of Merey and Pilon crude oil spilled from the M/T ALVENUS on 30 July 1984. Summary of analyses performed by Conoco laboratories.

Attribute/Chemical	Merey	Pilon
Specific gravity at 15.5°C	0.958	0.978
Total sulfur, wt %	2.5	2.7
Heavy metals, ppm		
Vanadium	265.0	265.0
Nickel	60.0	70.0
Copper	$<$0.5	$<$0.5
Fractional distillation, temperature cut range (°C)	% yield by weight	
C_1–C_5 gases	0.10	–
37.8 – 85.0	1.49	–
85.0 – 193.3	3.96	–
193.3 – 232.2	3.12	3.84
232.2 – 265.5	3.92	3.26
265.5 – 335.0	10.88	10.72
335.0 – 385.0	8.91	9.37
385.0 – 418.3	6.20	6.46
418.3 – 515.5	14.09	15.60
515.5 +	47.35	50.80

TABLE 2

Physical and chemical properties of weathered crude oil spilled from the M/T ALVENUS and collected from Jamaica Beach on 9 August 1984. Summary of analyses performed by Conoco laboratories.

Attribute/Chemical	Level
% wt	
Water	15.0
Solids	65.0
Oil	20.0
Analysis of oil component	
Specific gravity at 15.5°C	0.9745
% wt sulfur	2.6
Heavy metals, ppm	
Vanadium	280.0
Nickel	77.0
Copper	1.0

TABLE 3

Typical hydrocarbon constituents of crude oil.

Fraction	Distillation temperature (°C)	Carbon number
Gas	below 20	C_1–C_4
Petroleum ether	20–60	C_5–C_6
Ligroin (light naphtha)	60–100	C_6–C_7
Natural gasoline	40–205	C_5–C_{10} and cycloalkanes.
Kerosene	175–325	C_{12}–C_{18} and aromatics.
Gas oils	300–400	C_{16}–C_{25}
Residual oils - asphaltenes	above 400	Above C_{25}, long chains attached to cyclic hydrocarbons. Molecular weight = $>$20,000.

TABLE 4

Results of silica gel column chromatography conducted by University of Texas laboratories on the petroleum fraction of oil spilled from the M/T ALVENUS, and collected from Galveston Island seawall on 7 and 28 August 1984.

	Percent Composition	
Component	8-7-84	8-28-84
Sand and insolubles	40.70	52.00
Petroleum fractions		
Saturated hydrocarbons	14.83	12.84
Aromatic hydrocarbons	21.34	15.58
N, S, O compounds	6.50	4.86
Asphaltenes	16.60	15.17

slightly (Table 4). Based on qualitative observations of the gas-liquid chromatogram, low molecular weight aromatics such as benzene through phenanthrene were largely absent in the weathered crude oil. These observations and the increased level of sand and insolubles at progressively later dates indicated natural weathering processes were active during the spill.

Analyses of the fresh and weathered crude oils revealed high levels of vanadium (Tables 1 and 2). Vanadium content did not appear to decrease with weathering. This strongly indicated that vanadium compounds present in the weathered oil were fairly stable.

Surface water samples collected at the beach on 7 August and 8 September 1984 yielded oil levels of 1,190 mg/l and 43 mg/l, respectively.

Water quality parameters measured varied little throughout the experiment. Dissolved oxygen never fell below 6.2 mg/l in any container. Water temperature varied between 25 and 26°C. Salinity fluctuated between 34 and 37 ppt.

Live larvae and eggs were observed in all containers on 8 September 1984 at 1900 hours. Appreciable amounts of surface oil had accumulated in various containers of the 50, 100, 500, and 2,000 mg/l treatments (Table 5). On 9 September 1984 at 0735 hours, one to three dead larvae exhibiting spinal curvature were observed in replicates one and two of the 1,000-mg/l treatment. Dead and deformed larvae were also observed in replicate two of the 2,000-mg/l treatment and replicates two and three of the 1,000-mg/l treatment.

Mortality of red drum eggs and yolk-sac larvae varied erratically between treatments (Table 5). In addition, high mortality was observed in replicate one of the control exposure. No statistically significant differences in mortality occurred in any of the treatments. Visible sublethal effects were limited to abnormal spinal curvature and were infrequent and statistically insignificant across all concentrations (Table 6). The size of the yolk-sac larvae ranged between 1.7 and 2.8 mm NL and averaged 2.3 mm NL. There were no significant differences in size of larvae among treatments (Table 7).

TABLE 5

Percent mortality of red drum eggs and larvae after a 64-h exposure to six concentrations of weathered crude oil spilled from the M/T ALVENUS. S denotes visible surface oil observed.

Concentration	Replicate			
mg/l	1	2	3	mean
0	60	4	0	21
50	16^S	20	32	23
100	24	0^S	32	19
500	0	24^S	24	16
1,000	68	24	32	41
2,000	40^S	40^S	16^S	32

TABLE 6

Percent incidence of spinal curvature in surviving red drum yolk-sac larvae after a 64-h exposure to six concentrations of weathered crude oil spilled from the M/T ALVENUS. S denotes visible surface oil observed.

Concentration	Replicate			
mg/l	1	2	3	mean
0	0	0	0	0
50	0^S	0	6	2
100	0	0^S	0	0
500	4	5^S	0	3
1,000	0	0	0	0
2,000	0^S	0^S	0^S	0

TABLE 7

Notochord length range (RG, mm) and mean notochord length (NL, mm) of surviving yolk-sac larvae after a 64-h exposure to six concentrations of weathered crude oil spilled from the M/T ALVENUS. Grand mean is denoted by $\overline{NL}$. S denotes surface oil observed.

	Concentration mg/l					
Replicate	0	50	100	500	1000	2000
1						
RG	2.0–2.3	1.7–1.9	2.4–2.6	2.5–2.8	2.2–2.6	2.3–2.5
NL	2.1	1.8^S	2.5	2.7	2.4	2.4^S
2						
RG	2.3–2.5	2.2–2.4	2.1–2.3	2.3–2.5	2.2–2.3	2.3–2.4
NL	2.4	2.3	2.2^S	2.4^S	2.2	2.4^S
3						
RG	2.3–2.4	2.1–2.4	2.2–2.5	2.4–2.5	2.2–2.3	2.3–2.4
NL	2.3	2.3	2.4	2.5	2.2	2.4^S
Total						
$\overline{NL}$	2.3	2.1	2.4	2.5	2.3	2.4

DISCUSSION

The high mortality observed in one of the control replicates suggests that other factors, besides oil, may be affecting the survival of red drum eggs and larvae in these experiments. Hydrological variables monitored were well within the range necessary for optimum survival (Holt et al. 1981). Ammonia may have been a problem to larvae in oil-treated containers. Degradation of the oil by bacteria may have generated high concentrations of un-ionized ammonia. Concentrations as low as 0.55 mg/l of un-ionized ammonia have been shown to significantly increase mortality in larval red drum (Holt and Arnold 1983). However, low stocking densities and the use of eggs and yolk-sac larvae virtually eliminated the introduction of ammonia by metabolic end products and/or external food sources. Based on previous experience with the culture of red drum, the high mortality observed in the control replicates may have been caused by bacterial contamination (Holt and McCarty 1984).

The higher mean mortalities observed in the 1,000- and 2,000-mg/l treatments suggests that the oil may increase larval mortality at and exceeding these levels. However, the incidence of gross abnormalities was low in these containers. Based on our observations, the weathered crude oil exhibited low toxicity to red drum eggs and larvae. A static bioassay conducted with adult pinfish (*Lagodon rhomboides*) substantiates the relatively low toxicity of this weathered crude oil to estuarine fish (Spears 1984). The reported 48 hour LC_{50} was 19,500 mg/l of weathered crude oil. However, adult fish are generally more tolerant to pollutants than egg and larval stages (McKim 1977).

The reduced toxicity observed at low treatment levels is related to the chemical composition of the weathered oil and the metabolism of aromatic hydrocarbons by the egg and larval stages of fish. Petroleum crude oil is a complex mixture of hydrocarbons and associated inorganic compounds. Each of these chemicals exhibits its own associated toxicity to aquatic organisms. The acute toxicities of many of these compounds have been determined for various species, but little information exists on their synergistic effects. There is general agreement that the acute toxicity of crude oil is positively correlated with the aromatic hydrocarbon content. Aromatics are generally more toxic than cycloalkanes which are in turn more toxic than paraffins. Within each of these hydrocarbon groups the smaller molecules are generally more acutely toxic. The toxicity of aromatic hydrocarbons increases with increasing molecular size from benezene to phenanthrene, although the 4- and 5-ring aromatics are not acutely toxic (Davis et al. 1984). However, these 4- and 5-ring polynuclear aromatic hydrocarbons, such as benzo(α)pyrene, are known carcinogens (Malins and Hodgins 1981).

The crude oil spilled from the M/T ALVENUS was a heavy viscous type. The majority of lighter, toxic alkanes and cycloalkanes were absent from the oils. However, at least 21% of the crude oil was composed of aromatic hydrocarbons (Table 4). Based on qualitative observations of the gas-liquid chromatogram, the majority of these compounds were high molecular weight, water insoluble, polycyclic aromatic hydrocarbons (PAH). The majority of the water soluble and light hydrocarbons were probably lost during the initial 3 days of weathering at sea. These soluble phases usually include benzene and alkylbenzenes. However, the less soluble 2- and 3-ring aromatic compounds that remain are generally more acutely toxic (Anderson et al. 1974). As indicated by the gas-liquid chromatogram, the weathered crude oil did not contain a high percentage of these low molecular weight polycyclic aromatic hydrocarbons. Microbial and photochemical degradation at sea may partly account for this observation. Lee and Ryan (1983) reported that the half-lives of various PAHs were reduced to approximately 3 days during September (28°C) in a controlled microcosm experiment.

The structure of the red drum eggs and yolk-sac larvae may have also provided some protection against the adverse effects of petroleum hydrocarbons. Korn and Rice (1981) found that eggs of coho salmon (*Oncorhynchus kitsutch*) were more tolerant of aromatic hydrocarbons than alevins or fry. They suggested that the choroin, the protective membrane of the egg, prevented the rapid uptake of aromatic hydrocarbons present in the water. The amount of yolk also influenced sensitivity because aromatic hydrocarbons were selectively partitioned into the yolk, thus reducing their availability to the embryo until yolk absorption.

The elevated level of vanadium observed in the fresh and weathered crude oil was evidently nontoxic. The elemental form of vanadium is insoluble in water. However, some compounds such as vanadium pentoxide are soluble and have induced chronic toxicity at levels above 0.08 mg/l in larval freshwater flagfish (*Jordanella floridae*) (Holdway and Sprague 1979). Analyses of the weathered crude oil suggests that vanadium compounds were relatively stable and resistant to chemical and/or biological degradation.

Long-term secondary effects to planktonic food items of larval red drum may have occurred but would be difficult to quantify. Dahl et al. (1983) reported that growth rates of copepod populations declined in controlled medium-scaled ecosystems when exposed to a 5 mm layer of surface crude oil.

The short-term effects of the M/T ALVENUS oil spill on red drum eggs and larvae were difficult to determine, but were probably limited to increased mortality caused by the physical coating of eggs and larvae in the field by floating heavy crude oil. The initial chemical composition of the fresh crude oil and the seasonally warm weather contributed to the natural degradation of the more soluble toxic components. Based on the low mortality, infrequency of gross deformities, and the similar sizes and developmental stages of all surviving larvae observed in the bioassay, the weathered oil could be classified as relatively nontoxic.

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